

AUS DEM INSTITUT FÜR ZUCHTHYGIENE DER VETERINÄR-MEDIZINISCHEN
FAKULTÄT DER UNIVERSITÄT ZÜRICH

Direktor: Prof. Dr. K. Zerobin

A STUDY OF THE EFFECTS
OF GROUP SIZE AND POPULATION DENSITY
UPON SOME ASPECTS
OF THE REPRODUCTIVE PERFORMANCE OF
FEMALE FÜ-ALBINO MICE KEPT IN ALL-FEMALE GROUPS
DURING THE FIRST 14 DAYS OF PREGNANCY

INAUGURAL-DISSERTATION

zur Erlangung der Doktorwürde
der Veterinär-Medizinischen Fakultät
der Universität Zürich

vorgelegt von

ANTHONY W. ELLERY B. Vet. Med. M. R. C. V. S.

Tierarzt
aus England
in Magden AG

Genehmigt auf Antrag von:

Prof. Dr. K. Zerobin, Referent
Prof. Dr. R. Wyler, Korreferent

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1. INTRODUCTION

During the past 50 years, a substantial body of work has been published which clearly shows that high population density has a deleterious effect upon many reproductive parameters in mice.

Crew and Mirskaia (1931) compared the production of breeding groups of mice of various sizes housed in wooden boxes of 1250 cm². Using group sizes of 1 male with 1 female, 2 with 2, 4 with 4, 8 with 8 and 12 with 12, they found increasing plug: pregnancy ratios, decreasing fertility rates and decreasing litter size with increasing group size.

Retzlaff (1938) repeated Crew and Mirskaia's experiment with identical group and cage sizes, and found decreasing reproductive rates but not decreasing litter size with increasing group size.

Christian and Lemunyan (1958) established a group of 10 male and 10 female mice in a cage of 1460 cm². After 6 weeks, they sacrificed the females and compared their reproductive functions with those of 10 littermate females which had been paired monogamously. Uterine implantation scars were present in all females of both groups, but only 3 of the crowded females bore litters or ever appeared grossly pregnant, whereas all the segregated females bore litters. The number of implantations per female and the number of young per litter were significantly less in the crowded females. It was concluded that crowding results in increased intrauterine mortality and probably diminished fertility.

The deleterious effects of increasing population density upon reproduction have also been demonstrated in freely-growing populations of mice.

Brown (1953) found increasing post-natal mortality rates with increasing population density due to other mice interfering with

the nest-building of parturient females. He found that successful weaning ceased completely when this disturbance factor reached a certain level, and that this acted to limit further population growth.

Southwick (1955) reported that aggressiveness, gregariousness, intermingling of sexes, nest destruction and abnormal communal nesting all increase in intensity with increasing population density, and that all these factors apparently have a deleterious effect on post-natal litter survival.

Christian (1956) also studied reproductive responses to population size in mice from freely-growing populations, and concluded that there was a steady decline in birth-rate and in infant survival with increasing population size, until both approached zero.

Lloyd and Christian (1969) proposed that increasing population density causes inhibition of oestrus, foetal resorption and sudden increases in infant mortality in mice.

Lloyd (1975) studied two freely-growing populations of mice. In one, he found that population growth was limited mainly by decreasing birth rates, while in the other increasing mortality of infants and young adults were the major control factors.

It is thus evident that increasing population density induces mechanisms which act to slow down and later to arrest population growth.

Christian (1960a, 1961) proposed that the increasing social pressures of progressive population growth induce a physiologic adaptive response in mice of both sexes. The anterior pituitary gland increases its production of adrenocorticotrophic hormone (ACTH), and this leads to hyperplasia and hypertrophy of the adrenal cortex with increased production of corticosteroids, which in turn causes increased uterine mortality and decreased

fertility. Parallel to this, the anterior pituitary reduces its production of gonadotrophins (FSH, LH) with resultant suppression of spermatogenesis, oestrus and sex steroid production.

The question then arises as to whether this 'social pressure' is exerted by both sexes equally, or predominantly by one sex.

In their sexually symmetrical breeding populations, Crew and Mirskaia (1931) noted that the males fought 'desperately and without respite', and that the death rate among males was remarkably high, the commonest form of injury being partial or complete castration.

Uhrich (1938) compared the fighting behaviour of grouped male mice with that of grouped females. He concluded that fighting is very common between males, rare between the two sexes, and very rare among the females except for a few days after the introduction of a new female.

Brown (1953) found in his freely-growing mouse populations that a high percentage of males were killed as a result of fighting, and that many of the surviving males were in poor physical condition at the end of the experiment.

Southwick (1955) noted in his freely-growing populations that both males and females were seen to initiate fights, but that males were by far the more active aggressors, and that their aggression increased with increasing population density.

Reimer and Petras (1967) examined the mice in a population cage at the end of a period of 8 months' free growth, and found that 62.8 % of the males but only 1.8 % of the females had bite wounds.

As well as causing fighting, it has been demonstrated experimentally that the social stress of overcrowding leads to a decrease in male reproductive performance.

Christian (1955a) placed male mice in groups of 1, 4, 6, 8, 16 and 32 for a period of a week, and then killed them and weighed their reproductive organs. He found that the sizes of the preputial glands, seminal vesicles and testes were inversely related to population density.

In the same year (Christian, 1955b), he repeated this experiment with the same results, and a year later (Christian, 1956) he compared the weights of the reproductive organs of male mice from freely-growing populations of intermediate and high population density with those from segregated control animals. The organs were smallest in the males from high-density populations and largest in the segregated controls.

It was the purpose of the present study to examine the effects of 'social pressure' in all-female groups of mice by assessing the influence of group size and population density on some aspects of female reproductive performance, particularly during the first 14 days of pregnancy. It was therefore decided that no more than 1 male should be used per breeding group, thus eliminating fighting between males and social stress within the population resulting from this fighting.

The use of 1 male per breeding group was considered, but also rejected. According to Rugh (1968), strain differences in frequency of mating or in the time which must elapse before a male can achieve a second mating vary from 1 hour to 4 days. In breeding groups containing 1 male each but with different numbers of females, the frequency with which females come into oestrus and thus the frequency with which the males are likely to mate are not the same in all groups. This might well lead to variations in male reproductive performance (e.g. semen quality) causing differences in the values of reproductive parameters measured in the females.

Were an increase in plug: pregnancy ratios to be found in the groups with the highest numbers of females, for example, it

would not be clear whether this effect was due to reduced female reproductive performance or to a reduction of fertility in the males resulting from an abnormally high mating frequency.

It was therefore decided that the purposes of the present study were best served in that the groups should contain only female mice, and that these females should be removed from their groups to be mated under identical conditions, and returned to their groups immediately afterwards.

In this way it was possible to examine whether population density in an all-female population exerts an effect, deleterious or beneficial, on various reproductive parameters of female mice. A wide range of group size/cage size combinations were used to allow any effect to be quantified, and the relative importance of the two components of population density, the number of animals in the group and the total floor area available, to be compared.

2. MATERIALS AND METHODS

2.1 Animals

The F $\ddot{u}$ -Albino mice used in this study were bred in an SPF (Specified Pathogen Free) unit in the Institute of Biological and Medical Research Ltd., CH-4414 F $\ddot{u}$ llinsdorf, Switzerland. This outbred stock is bred according to the 12 unit system of Poiley (1960). The mice in the SPF unit have a hygienic status equivalent to the MRC Category **** (Medical Research Council, 1974), this status being monitored according to the methods recommended by the Society for Laboratory Animal Science (GV-SOLAS, 1977).

2.2 Housing and care

The study was performed in an animal room in the Institute of Biological and Medical Research. This room has a floor area of 25 m² and was maintained at a temperature of 21 $\pm$ 2 $^{\circ}$ C with a relative humidity of 40 - 60 % and approximately 15 air changes per hour. The room was lit by a mixture of natural and artificial light with a light:darkness cycle of 15:9 hours. The study was performed from August to December, 1978.

The mice used in the study were transported from the SPF unit into this room in filter cages. Caging materials, food, bedding and other materials entering the room were first sterilized or disinfected, and animal caretakers working within the room wore additional protective clothing (caps and gloves) as well as their usual working uniforms.

Standard Makrolon cages (Types I, II and III) with sawdust bedding were used throughout the study and were changed once weekly.

2.3 Diet and feeding

The mice were given autoclaved Nafag 857 DVOD pellets (autoclave programme: 121°C, 20 minutes) and ozonized tap water in plastic drinking bottles. Both food and water were available ad libitum.

2.4 Experimental plan

306 virgin female SPF F₁-Albino mice aged 28 days were transported from the SPF unit into the experimental room where they were randomized into 10 groups, 6 cages per group, as shown below:

Group	No. of ♀♀ per cage	Cage number	Total ♀♀ per group	Cage type	Cage area cm ²	Cage area per ♀ cm ²
1	1	1 - 6	6	I	180	180
2	2	7 - 12	12	I	180	90
3	4	13 - 18	24	I	180	45
4	2	19 - 24	12	II	360	180
5	4	25 - 30	24	II	360	90
6	8	31 - 36	48	II	360	45
7	2	37 - 42	12	III	770	385
8	4	43 - 48	24	III	770	193
9	8	49 - 54	48	III	770	96
10	16	55 - 60	96	III	770	48

Using randomization tables, 1 female per cage was selected and marked on the back with picric acid.

On the same day, 70 virgin male SPF F₁-Albino mice aged 28 days were also brought from the SPF unit into the experimental room. They were placed singly in Makrolon Type I cages numbered 1-70.

At the age of 49 days (=Day 0, three weeks after the start of the experiment) each marked female was taken from its group cage and placed in the cage of the male with the corresponding number (1 → 1, 2 → 2 and so on up to 60 → 60). These pairings were established between 07.30 and 08.00 h.

On the same afternoon between 15.30 and 16.15 h, each marked female was examined for the presence of a vaginal plug. Females with plugs were immediately returned to their original group cages.

On the following 4 mornings (Day 1 to Day 4) between 07.00 and 07.45 h, the marked females which were still with the males were re-examined for the presence of vaginal plugs. Each female showing a vaginal plug was immediately returned to its group cage. Females which had shown no plugs were also returned to their group cages on Day 4.

The day on which each plug was found was recorded.

Exactly 14 days after its plug had been found, each marked female was killed (ether narcosis followed by cervical dislocation) and dissected. Marked females which had shown no vaginal plugs were dissected on Day 18 (= 14 days after Day 4).

The following parameters were recorded for each dissected female:

- group cage number
- pairing cage number (= number of stud male)
- day on which vaginal plug was found (Day 0 - Day 4)
- date of dissection
- time of dissection
- bodyweight of dissected female, measured to the nearest mg immediately after death using a Mettler PL 200 balance.

NOTE: Bodyweights, organ weights and foetus and placenta weights were not recorded for non-pregnant females, nor for females which had not shown vaginal plugs (unknown duration of pregnancy).

- weight of right ovary
- weight of left ovary
- weight of left adrenal gland

The 3 organs listed above were carefully dissected out and weighed to the nearest 0.1 mg using a Mettler H 14 balance. The adrenal gland was discarded, and the ovaries placed on a moist filter paper in a Petri dish and kept for the subsequent counting of corpora lutea.

- weight of uterus

The uterus was removed and weighed to the nearest mg using a Mettler PL 200 balance. The uterus was then incised at the cervical end of one horn and the foetus nearest the cervix removed within its amnion and with placenta attached. Using fine scissors, the amnion was removed from foetus and placenta. The foetus and placenta were gently blotted dry and weighed immediately.

- weight of living foetus (to nearest mg, using a Mettler PL 200 balance)
- weight of placenta of living foetus (to nearest mg, using a Mettler PL 200 balance)

The other foetuses and placentae in the uterus were removed and weighed in the same way, proceeding from the cervix towards the apex of one horn, and then from the cervix to the apex of the second.

- weight of dead implant (to nearest mg, using a Mettler PL 200 balance)
- number of living foetuses
- number of dead implants

Throughout the study, the left and right horns of the uterus were dissected first alternately.

- number of corpora lutea

The corpora lutea were counted under a dissection microscope within 30 minutes of the removal of the ovaries.

The experiment was repeated as described 9 times, giving a total of 10 experimental series in the whole study. New females, but the same stud males, were used in each series. Up to and including the 3rd series, the males were paired with the marked females of the corresponding group cages (1 with 1, 2 with 2, and so on). From the 4th series onwards, pairings were arranged in such a way that no male was used in any one group more than once, and not at all in the group in which it had been used during series 1, 2 and 3. This set-up was designed to facilitate a comparison of the reproductive performance of male mice during their first 3 parities. The results of the comparison are not included in this study.

3. RESULTS

3.1 Results

The results of the study are shown in Figures 1 - 16. Figures 1 - 6 each consist of two parts, a) and b). In each case, table a) shows the results obtained for each of the 10 experimental groups, averages and standard errors having been calculated where appropriate. Table b) groups the results according to cage type (cage size), group size and population density as follows:

Cage type	I	Groups 1, 2, 3
	II	4, 5, 6
	III	7, 8, 9, 10
Group size	1	1
	2	2, 4, 7
	4	3, 5, 8
	8	6, 9
	16	10
Population density (cm ² per female)	45	3, 6, 10
	90	2, 5, 9
	180	1, 4, 8
	385	7

For the purpose of the population density groupings, Makrolon III cages were assumed to have twice the floor area of Makrolon II cages (in fact the ratio is 2,14).

Figures 7 - 12 show how various parameters varied according to the time of day at which the section was performed, and Figures 13 - 16 show how the day after pairing on which mating occurred affected various parameters.

During the whole study, a total of 590 female mice were dissected. 515 were found to be pregnant at dissection with a total of more than 6,000 fetuses.

Depending on the parameter being considered, more or less of the total number of females were included in the assessment. Which females were included is shown in brackets on each Figure.

Thus, for the assessment of the average numbers of corpora lutea, living fetuses and dead implants, only females were considered in which plugs had been found, and which were therefore known to be 14 days pregnant. For the assessment of average weights, only those females were included which were dissected prior to the lunch break at ca. 11.15 h. By eliminating females which were dissected during the afternoon, any influence of circadian rhythmic variations in weight and in growth of the fetuses (see Figures 7 - 12) were kept to a minimum.

FIGURE 1a Day after pairing on which vaginal plug found
(all pregnant females in which plugs were found)

	Group										All Groups
	1	2	3	4	5	6	7	8	9	10	
Day 0 number of plugs (% of total)	7 (17.9)	4 (8.2)	8 (17.0)	4 (8.5)	5 (10.9)	3 (6.3)	4 (8.3)	3 (7.1)	6 (14.0)	3 (7.3)	47 (10.4)
Day 1 number of plugs (% of total)	6 (15.4)	7 (14.3)	6 (12.8)	9 (19.1)	4 (8.7)	11 (22.9)	11 (22.9)	6 (14.3)	8 (18.6)	6 (14.6)	74 (16.4)
Day 2 number of plugs (% of total)	4 (10.3)	14 (28.6)	9 (19.1)	11 (23.4)	10 (21.7)	6 (12.5)	10 (20.8)	4 (9.5)	4 (9.3)	9 (22.0)	81 (18.0)
Day 3 number of plugs (% of total)	15 (38.5)	19 (38.8)	20 (42.6)	17 (36.2)	19 (41.3)	20 (41.7)	18 (37.5)	22 (52.4)	20 (46.5)	18 (43.9)	188 (41.8)
Day 4 number of plugs (% of total)	7 (17.9)	5 (10.2)	4 (8.5)	6 (12.8)	8 (17.4)	8 (16.7)	5 (10.4)	7 (16.7)	5 (11.6)	5 (12.2)	60 (13.3)
Total plugs	39	49	47	47	46	48	48	42	43	41	450

FIGURE 1b Day after pairing on which vaginal plug found
(all pregnant females in which plugs were found)

	Cage type			Group size					cm ² per female			
	I	II	III	1	2	4	8	16	45	90	180	385
Day 0 number of plugs (% of total)	19 (14.1)	12 (8.5)	16 (9.2)	7 (17.9)	12 (8.3)	16 (11.9)	9 (9.9)	3 (7.3)	14 (10.3)	15 (10.9)	14 (10.9)	4 (8.3)
Day 1 number of plugs (% of total)	19 (14.1)	24 (17.0)	31 (17.8)	6 (15.4)	27 (18.8)	16 (11.9)	19 (20.9)	6 (14.6)	23 (16.9)	19 (13.8)	21 (16.4)	11 (22.9)
Day 2 number of plugs (% of total)	27 (20.0)	27 (19.1)	27 (15.5)	4 (10.3)	35 (24.3)	23 (17.0)	10 (11.0)	9 (22.0)	24 (17.6)	28 (20.3)	19 (14.8)	10 (20.8)
Day 3 number of plugs (% of total)	54 (40.0)	56 (39.7)	78 (44.8)	15 (38.5)	54 (37.5)	61 (45.2)	40 (44.0)	18 (43.9)	58 (42.6)	58 (42.0)	54 (42.2)	18 (37.5)
Day 4 number of plugs (% of total)	16 (11.9)	22 (15.6)	22 (12.6)	7 (17.9)	16 (11.1)	19 (14.1)	13 (14.3)	5 (12.2)	17 (12.5)	18 (13.0)	20 (15.6)	5 (10.4)
Total plugs	135	141	174	39	144	135	91	41	136	138	128	48

FIGURE 2a Plug and pregnancy rates
(all females)

	Group										All Groups
	1	2	3	4	5	6	7	8	9	10	
Total females	58	60	57	58	60	59	59	60	59	60	590
Number with plug (% of total)	45 (77.6)	51 (85.0)	50 (87.7)	48 (82.8)	52 (86.7)	51 (86.4)	49 (83.1)	46 (76.7)	46 (78.0)	44 (73.3)	482 (81.7)
Number becoming pregnant (% of total)	49 (84.5)	57 (95.0)	52 (91.2)	51 (87.9)	52 (86.7)	53 (89.8)	52 (88.1)	51 (85.0)	49 (83.1)	49 (81.7)	515 (87.3)
Number with plug becoming pregnant (% of number with plug)	40 (88.9)	49 (96.1)	47 (94.0)	47 (97.9)	46 (88.5)	48 (94.1)	48 (98.0)	43 (93.5)	43 (93.5)	41 (93.2)	452 (93.8)

No significant group effects (see Section 3.2)

FIGURE 2b Plug and pregnancy rates
(all females)

	Cage type			Group size					cm ² per female			
	I	II	III	1	2	4	8	16	45	90	180	385
Total females	175	177	238	58	177	177	118	60	176	179	176	385
Number with plug (% of total)	146 (83.4)	151 (85.3)	185 (77.7)	45 (77.6)	148 (83.6)	148 (83.6)	97 (82.2)	44 (73.3)	145 (82.4)	149 (83.2)	139 (79.0)	49 (83.1)
Number becoming pregnant (% of total)	158 (90.3)	156 (88.1)	201 (84.5)	49 (84.5)	160 (90.4)	155 (87.6)	102 (86.4)	49 (81.7)	154 (87.5)	158 (88.3)	151 (85.8)	52 (88.1)
Number with plug becoming pregnant (% of number with plug)	136 (93.2)	141 (93.4)	175 (94.6)	40 (88.9)	144 (97.3)	136 (91.9)	91 (93.8)	41 (93.2)	136 (93.8)	138 (92.6)	130 (93.5)	48 (98.0)

No significant group effects (see Section 3.2)

FIGURE 3a

Bodyweight, uterus weight, uterus weight as % of bodyweight, bodyweight minus uterus weight (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

Group	n	Bodyweight of pregnant females (g)		Uterus weight (g)		Uterus weight as % bodyweight		Bodyweight minus uterus weight (g)	
		Average	S.E.	Average	S.E.	Average	S.E.	Average	S.E.
1	33	49.87	0.74	8.86	0.23	17.77	0.38	41.01	0.64
2	36	50.41	1.07	9.42	0.39	18.59	0.59	40.98	0.85
3	36	50.30	0.94	9.18	0.34	18.17	0.52	41.12	0.75
4	34	53.06	0.91	9.66	0.26	18.24	0.44	43.40	0.82
5	34	49.32	0.94	8.88	0.31	17.94	0.47	40.44	0.75
6	39	50.37	0.80	9.22	0.25	18.33	0.45	41.15	0.71
7	34	49.85	0.77	8.96	0.34	17.94	0.62	40.89	0.67
8	30	49.71	1.11	9.22	0.35	18.47	0.58	40.49	0.89
9	33	50.89	0.99	9.24	0.25	18.15	0.35	41.65	0.81
10	35	50.12	0.83	8.97	0.30	17.87	0.49	41.14	0.69
Total	344	50.40	0.29	9.16	0.10	18.15	0.16	41.23	0.24
Anova:									
Calculated error probability		0.2649		0.7366		0.9775		0.3115	

No significant group effects (see Section 3.2)

FIGURE 3b Average bodyweight, uterus weight, uterus weight as % of bodyweight, bodyweight minus uterus weight (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

		n	Bodyweight of pregnant female (g)	Uterus weight (g)	Uterus weight as % bodyweight	Bodyweight minus uterus weight (g)
Cage type	I	105	50.20	9.16	18.19	41.04
	II	107	50.89	9.25	18.18	41.64
	III	132	50.15	9.09	18.09	41.06
Anova: calculated error probability			0.5206	0.7963	0.9626	0.5302
Group size	1	33	49.87	8.86	17.77	41.01
	2	104	51.09	9.35	18.26	41.74
	4	100	49.79	9.09	18.18	40.70
	8	72	50.61	9.23	18.25	41.38
	16	35	50.12	8.97	17.87	41.14
Anova: calculated error probability			0.4800	0.6143	0.8862	0.5741
Population density (cm ² per female)	45	110	50.27	9.13	18.13	41.14
	90	103	50.20	9.19	18.23	41.02
	180	97	50.94	9.25	18.15	41.69
	385	34	49.85	8.96	17.94	40.89
Anova: calculated error probability			0.6786	0.8673	0.9660	0.6897

No significant group effects (see Section 3.2)

FIGURE 4a Total ovary weight, total ovary weight as $\frac{0}{1000}$ of bodyweight, weight of left adrenal gland, weight of left adrenal gland as $\frac{0}{1000}$ of bodyweight (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

Group	n	Weight of both ovaries (mg)		Ovary weight as $\frac{0}{1000}$ bodyweight		Weight of left adrenal gland(mg)		Adrenal weight as $\frac{0}{1000}$ bodyweight	
		Average	S.E.	Average	S.E.	Average	S.E.	Average	S.E.
1	33	19.32	0.78	3.86	0.13	5.85	0.23	1.19	0.05
2	36	19.58	0.69	3.88	0.11	5.59	0.20	1.12	0.04
3	36	18.74	0.71	3.70	0.11	5.43	0.20	1.08	0.04
4	34	19.65	0.62	3.71	0.10	5.50	0.20	1.05	0.04
5	34	18.60	0.46	3.78	0.08	5.57	0.17	1.13	0.03
6	39	19.16	0.57	3.81	0.10	5.65	0.18	1.13	0.04
7	34	19.42	0.59	3.91	0.12	5.65	0.23	1.14	0.05
8	30	18.53	0.66	3.74	0.11	5.49	0.22	1.13	0.06
9	33	18.00	0.54	3.56	0.09	5.26	0.20	1.04	0.04
10	35	18.19	0.51	3.64	0.10	5.23	0.19	1.05	0.04
Total	344	18.93	0.20	3.76	0.03	5.52	0.06	1.11	0.01

Anova: Calculated error probability	0.5240	0.3406	0.5720	0.2814
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No significant group effects (see Section 3.2)

FIGURE 4b Average total ovary weight, total ovary weight as $\frac{0}{1000}$ of bodyweight, weight of left adrenal gland, weight of left adrenal gland as $\frac{0}{1000}$ of bodyweight (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

Cage type		n	Weight of both ovaries (mg)	Ovary weight as $\frac{0}{1000}$ bodyweight	Weight of left adrenal gland (mg)	Adrenal weight as $\frac{0}{1000}$ bodyweight
	I	105	19.2	3.82	5.62	1.13
	II	107	19.1	3.77	5.58	1.10
	III	132	18.5	3.71	5.40	1.09
Anova: calculated error probability			0.2959	0.4182	0.3336	0.5281
Group size	1	33	19.3	3.86	5.85	1.19
	2	104	19.6	3.83	5.58	1.10
	4	100	18.6	3.74	5.49	1.11
	8	72	18.6	3.69	5.47	1.09
	16	35	18.2	3.64	5.23	1.05
Anova: calculated error probability			0.1774	0.3251	0.2686	0.2746
Population density (cm^2 per female)	45	110	18.7	3.72	5.44	1.09
	90	103	18.8	3.75	5.48	1.10
	180	97	19.2	3.77	5.62	1.12
	385	34	19.4	3.91	5.65	1.14
Anova: calculated error probability			0.6071	0.4874	0.6423	0.6776

No significant group effects (see Section 3.2)

FIGURE 5a

Number of corpora lutea, number of living foetuses, total number of implants (= living foetuses + dead implants), number of corpora lutea minus total implants
(all 14-days-pregnant females)

Group	n	Number of corpora lutea		Number of living foetuses		Total number of implants		Corpora lutea minus total implants	
		Average	S.E.	Average	S.E.	Average	S.E.	Average	S.E.
1	39	13.49	0.37	11.38	0.33	12.03	0.32	1.46	0.33
2	49	13.49	0.38	11.84	0.37	12.78	0.37	0.71	0.23
3	45	13.67	0.36	12.27	0.41	13.09	0.33	0.58	0.18
4	47	13.94	0.33	12.43	0.28	13.17	0.30	0.77	0.24
5	45	13.27	0.30	11.69	0.35	12.40	0.33	0.87	0.19
6	48	13.75	0.32	12.04	0.31	12.63	0.26	1.13	0.23
7	48	13.56	0.32	11.40	0.42	12.40	0.38	1.17	0.24
8	42	13.33	0.35	11.64	0.44	12.26	0.38	1.07	0.24
9	42	13.48	0.34	11.90	0.29	12.74	0.25	0.74	0.25
10	41	13.76	0.26	11.78	0.37	12.90	0.32	0.85	0.22
Total	446	13.58	0.11	11.85	0.12	12.65	0.10	0.93	0.07

Anova: Calculated error probability	0.9438	0.5474	0.2890	0.2775
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No significant group effects (see Section 3.2)

FIGURE 5b Average number of corpora lutea, number of living foetuses, total number of implants (= living foetuses + dead implants), number of corpora lutea minus total implants (all 14-days-pregnant females)

Cage type		n	Number of corpora lutea	Number of living foetuses	Total number of implants	Corpora lutea minus total implants
	I	133	13.55	11.85	12.66	0.89
	II	140	13.66	12.06	12.74	0.92
	III	173	13.53	11.67	12.57	0.97
Anova: calculated error probability			0.8717	0.3775	0.7940	0.9111
Group size	1	39	13.49	11.38	12.03	1.46
	2	144	13.66	11.88	12.78	0.88
	4	132	13.42	11.87	12.59	0.83
	8	90	13.62	11.98	12.68	0.94
	16	41	13.76	11.78	12.90	0.85
Anova: calculated error probability			0.8801	0.7849	0.3735	0.2746
Population density (cm ² per female)	45	134	13.72	12.04	12.87	0.86
	90	136	13.41	11.81	12.64	0.77
	180	128	13.60	11.85	12.52	1.08
	385	48	13.56	11.40	12.40	1.17
Anova: calculated error probability			0.7165	0.4762	0.5055	0.2813

No significant group effects (see Section 3.2)

FIGURE 6a

Weight of living foetus, weight of placenta of living foetus, sum of weights of living foetuses (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

Group	n	Weight of living foetus (mg)		Weight of placenta (mg)		Sum of weights of living foetuses (g)	
		Average	S.E.	Average	S.E.	Average	S.E.
1	33	331.8	6.03	121.3	2.27	3.763	0.12
2	36	328.3	6.05	122.1	1.77	3.981	0.18
3	36	310.9	5.39	115.3	2.35	3.932	0.17
4	34	329.0	4.99	119.0	1.79	4.162	0.13
5	34	322.2	5.66	118.7	2.11	3.770	0.14
6	39	321.8	5.06	117.1	1.98	3.949	0.12
7	34	316.3	5.86	120.8	2.46	3.683	0.17
8	30	330.3	5.40	118.4	2.19	3.997	0.17
9	33	324.5	4.06	120.9	2.52	3.881	0.11
10	35	322.9	5.87	120.7	1.70	3.800	0.15
Total	344	323.6	1.96	119.4	0.76	3.892	0.05

Variance analysis: calculated error probability	0.3684	0.6226	0.5706
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No significant group effects (see Section 3.2)

FIGURE 6b

Average weight of living foetus, weight of placenta of living foetus, sum of weights of living foetuses (all 14-day-pregnant females dissected between 07.00 and 11.15 h)

Cage type		n	Weight of living foetus (mg)	Weight of placenta (mg)	Sum of weights of living foetuses (g)
	I	105	323.4	119.5	3.90
	II	107	324.2	118.2	3.96
	III	132	323.3	120.3	3.84
Anova: calculated error probability			0.9794	0.5357	0.5627
Group size	1	33	331.8	121.3	37.6
	2	104	324.6	120.7	39.4
	4	100	320.6	117.4	39.0
	8	72	323.1	118.9	39.2
	16	35	322.9	120.7	38.0
Anova: calculated error probability			0.6613	0.4366	0.8349
Population density (cm ² per female)	45	110	318.6	117.7	39.0
	90	103	325.1	120.6	38.8
	180	97	330.3	119.6	39.8
	385	34	316.3	120.8	36.8
Anova: calculated error probability			0.0771	0.4247	0.4370

No significant group effects (see Section 3.2)

FIGURE 7 Relation between bodyweight and time of day
(all 14-days-pregnant females dissected between
07.30 and 11.30 h)



Time of Day	07.30 -08.00	08.00 -08.30	08.30 -09.00	09.00 -09.30	09.30 -10.00	10.00 -10.30	10.30 -11.00	11.00 -11.30
n	31	61	66	45	52	44	34	19
Average bodyweight (g)	51.97	51.19	50.92	50.20	49.80	49.51	48.95	48.25
S.E.	0.96	0.66	0.64	0.84	0.79	0.81	0.95	1.36

Significant decrease of bodyweight with time ($p = 0.0005$, see Section 3.2)

FIGURE 8 Relation between uterus weight and time of day
(all 14-days-pregnant females dissected between
07.30 and 11.30 h)



Time of day	07.30 -08.00	08.00 -08.30	08.30 -09.00	09.00 -09.30	09.30 -10.00	10.00 -10.30	10.30 -11.00	11.00 -11.30
n	31	61	66	45	52	44	34	19
Average uterusweight (g)	8.90	8.83	9.32	9.04	9.22	9.57	8.97	9.46
S.E.	0.37	0.25	0.19	0.26	0.27	0.27	0.31	0.37

Significant increase of uterus weight with time ($p = 0.0457$, see Section 3.2)

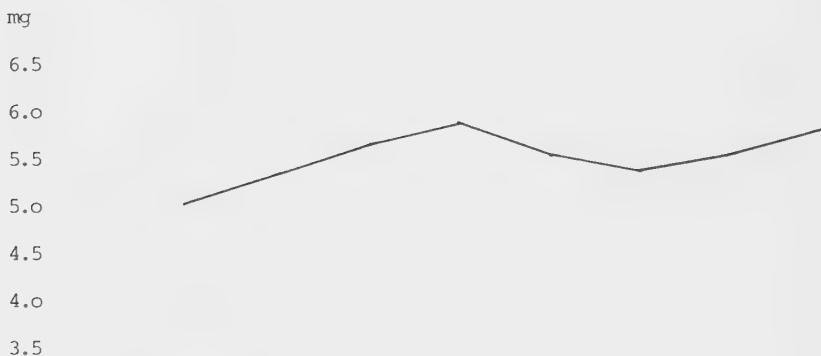
FIGURE 9 Relation between sum of weights of ovaries and time of day (all 14-days-pregnant females dissected between 07.00 and 11.30 h)



Time of day	07.30 -08.00	08.00 -08.30	08.30 -09.00	09.00 -09.30	09.30 -10.00	10.00 -10.30	10.30 -11.00	11.00 -11.30
n	31	61	66	45	52	44	34	19
Average sum of weights of both ovaries (mg)	17.8	19.0	18.9	19.0	18.7	19.0	19.6	19.0
S.E.	0.54	0.48	0.43	0.55	0.50	0.58	0.67	0.95

No significant relationship between ovary weight and time ($p = 0.2520$,
see Section 3.2)

FIGURE 10 Relation between weight of left adrenal gland and time of day (all 14-days-pregnant females dissected between 07.00 and 11.30 h)



Time of day	07.30 -08.00	08.00 -08.30	08.30 -09.00	09.00 -09.30	09.30 -10.00	10.00 -10.30	10.30 -11.00	11.00 -11.30
n	31	61	66	45	52	44	34	19
Average weight of left adrenal gland (mg)	5.04	5.31	5.63	5.89	5.59	5.33	5.59	5.79
S.E.	0.20	0.16	0.12	0.21	0.16	0.17	0.20	0.29

Significant increase of adrenal gland weight with time ($p = 0.0305$,
see Section 3.2)

FIGURE 11 Relation between foetus weight and time of day
(all 14-days-pregnant females dissected between
07.30 and 11.30 h)



Time of day	07.30 -08.00	08.00 -08.30	08.30 -09.00	09.00 -09.30	09.30 -10.00	10.00 -10.30	10.30 -11.00	11.00 -11.30
n	31	61	66	45	52	44	34	19
Average foetus weight (mg)	321.8	313.9	323.5	321.2	323.7	330.6	331.3	333.1
S.E.	6.02	6.86	3.63	5.22	4.39	4.71	5.23	7.17

Significant increase of foetus weight with time ($p = 0.0198$, see Section 3.2)

FIGURE 12 Relation between placenta weight and time of day
(all 14-days-pregnant females dissected between
07.30 and 11.30)



Time of day	07.30-08.00	08.00-08.30	08.30-09.00	09.00-09.30	09.30-10.00	10.00-10.30	10.30-11.00	11.00-11.30
n	31	61	66	45	52	44	34	19
Average placenta weight (mg)	119.4	117.2	117.0	118.8	120.5	120.2	122.1	124.2
S.E.	2.49	2.47	1.45	1.85	1.96	1.55	1.93	3.91

Significant increase of placenta weight with time ($p = 0.0305$, see Section 3.2)

FIGURE 13

Average bodyweight, uterus weight, uterus weight as % of bodyweight, bodyweight minus uterus weight (all 14-day-pregnant females dissected between 07.00 and 11.15 h)

Day after pairing on which vaginal plug found	n	Bodyweight of pregnant female (g)	Uterus weight (g)	Uterus weight as % bodyweight	Bodyweight minus uterus weight (g)
0	42	48.61	8.17	16.77	40.44
1	51	50.61	9.47	18.76	41.14
2	65	51.67	9.55	18.42	42.12
3	142	50.38	9.23	18.28	41.15
4	44	50.01	8.96	17.91	41.05
Total	344	50.40	9.16	18.15	41.23

Anova: calculated error probability	0.0739	0.0011	0.0005	0.4110
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Significant differences between plug-days (t-tests)	-	1 > 0 (0.0005) 2 > 0 (0.0001) 3 > 0 (0.0007) 4 > 0 (0.0378)	1 > 0 (0.0010) 2 > 0 (0.0041) 3 > 0 (0.0030)	-
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see Section 3.2

FIGURE 14 Average total ovary weight, total ovary weight as $\frac{0}{1000}$ of bodyweight, weight of left adrenal gland, weight of left adrenal gland as $\frac{0}{1000}$ of bodyweight (all 14-days-pregnant females dissected between 07.00 and 11.15 h)

Day after pairing on which vaginal plug found	n	Weight of both ovaries (mg)	Ovary weight as $\frac{0}{1000}$ bodyweight	Weight of left adrenal gland (mg)	Adrenal weight as $\frac{0}{1000}$ of bodyweight
0	42	17.81	3.67	5.31	1.10
1	51	17.76	3.50	5.36	1.07
2	65	19.02	3.69	5.30	1.03
3	142	19.82	3.94	5.75	1.15
4	44	18.34	3.67	5.50	1.12
Total	344	18.93	3.76	5.52	1.11

Anova: calculated error probability	0.0005	0.0001	0.0386	0.0265
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Significant differences between plug-days (t-tests)	3 > 0 (0.0013) 3 > 1 (0.0004) 3 > 4 (0.0114)	3 > 0 (0.0096) 3 > 1 (0.0001) 3 > 2 (0.0046) 3 > 4 (0.0082)	3 > 0 (0.0337) 3 > 1 (0.0399) 3 > 2 (0.0100)	3 > 1 (0.0436) 3 > 2 (0.0020)
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FIGURE 15 Average number of corpora lutea, number of living foetuses, total number of implants (= living foetuses + dead implants), number of corpora lutea minus total implants (all 14-days-pregnant females)

Day after pairing on which vaginal plug found	n	Number of corpora lutea	Number of living foetuses	Total number of implants	Corpora lutea minus total implants
0	47	13.13	11.17	12.11	1.02
1	74	13.73	12.18	12.86	0.86
2	78	14.12	12.46	13.26	0.86
3	187	13.74	11.89	12.78	0.96
4	60	12.52	11.02	11.60	0.92
Total	446	13.58	11.85	12.65	0.93

Anova: calculated error probability	0.0002	0.0018	0.0001	0.9716
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Significant differences between plug-days (t-tests)	1 > 4 (0.0015) 2 > 4 (0.0001) 3 > 4 (0.0002) 2 > 0 (0.0143)	1 > 4 (0.0057) 2 > 4 (0.0006) 3 > 4 (0.0142) 2 > 0 (0.0038)	1 > 4 (0.0009) 2 > 4 (0.0001) 3 > 4 (0.0003) 2 > 0 (0.0041)	-
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FIGURE 16 Average weight of living foetus, weight of placenta of living foetus, sum of weights of living foetuses (all 14-day-pregnant females dissected between 07.00 and 11.15 h)

Day after pairing on which vaginal plug found	n	Weight of living foetus (mg)	Weight of placenta (mg)	Sum of weights of living foetuses (g)
0	42	302.1	115.7	3.417
1	51	332.1	121.5	4.110
2	65	316.9	119.1	4.012
3	142	325.1	119.6	3.895
4	44	339.4	120.3	3.911
Total	344	323.6	119.4	3.892

Anova: calculated error probability	0.0000	0.3684	0.0025
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Significant differences between plug-days (t-tests)	1 > 0 (0.0001) 2 > 0 (0.0359) 3 > 0 (0.0003) 4 > 0 (0.0000) 1 > 2 (0.0220) 4 > 2 (0.0013) 4 > 3 (0.0206)	-	1 > 0 (0.0002) 2 > 0 (0.0007) 3 > 0 (0.0021) 4 > 0 (0.0094)
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see Section 3.2

3.2 Statistical assessment of results

The significance of the results was investigated using the following test procedures, whereby a significance level of $p = 0.05$ was selected.

Figures 2 a) and b)

Group effects were investigated by calculating the Chi-Square values for the appropriate 2×10 contingency tables (Ostle, 1972).

Figures 3-6, a) and b), 13-16

Group and 'plug-day' effects were investigated using one-way analysis of variance (ANOVA). Where this analysis produced significant results ($p = \leq 0.05$, as in Figures 13-16), then all groups were compared individually with each other using t-tests (Bennett and Franklin, 1954).

Figures 7-12

Time effects were investigated by calculating the Kendall rank correlation coefficient for time of day and weight (Kendall, 1970).

4. DISCUSSION

4.1 Effects of cage size, group size and population density on various reproductive parameters

4.1.1 Oestrous cycle (Fig. 1)

It has frequently been reported that grouping female mice leads to suppression or inhibition of oestrous cycles (Lee and Boot, 1955, 1956; Whitten, 1957, 1959; Lamond, 1959; Bronson and Chapman, 1968; Champlin, 1971; Ryan and Schwarz, 1977). Adding a male to the group, or removing the females from the group and pairing them with males, leads to the 'Whitten Effect' (Whitten, 1956, 1966), whereby ovulation is synchronized with less matings than expected on the 1st, 2nd and 4th nights after pairing, and matings on the 3rd night greatly exceeding expectations.

Whitten (1959) found this phenomenon to be relatively less pronounced in females housed singly prior to pairing than in females which had been grouped.

It has further been demonstrated that male urine will induce the 'Whitten Effect' in grouped females even in the absence of the male (Marsden and Bronson, 1965), while individually housed females show oestrus suppression typical of grouped females if placed in dirty cages which were occupied by females (Chaplin, 1971).

In the present study, the 'Whitten Effect' was clearly seen in all Groups, including Group 1 (isolated females). There was no evidence to suggest that the cage sizes, group sizes or population densities used in this study modify the intensity of the effect.

4.1.2 Mating (Fig. 2)

It has been demonstrated that a high proportion (87 %) of previously grouped female mice mate during a pairing period of

4 days (Whitten, 1956). This proportion has been shown to be higher for previously grouped females than for previously isolated females (89 % compared with 75 %), possibly due to a proportion of the previously isolated females not mating on the 1st night after pairing despite being in oestrus (Whitten, 1959).

Before looking at the result of the present study, it is important to emphasize the fact that all females in the study were paired monogamously, separate from their groups. Any effect exerted by population density at the moment of mating would not have been detected.

In the present study, 82.1 % of the paired females from Groups 2-10 (previously grouped females) showed vaginal plugs during the 4 days of pairing compared with 77.6 % of the previously isolated females from Group 1. This difference was not significant.

No effect of population density upon the proportion of females mating during the subsequent pairing period was detected.

4.1.3 Fertility (Fig. 2)

In the context of this present study, fertility has been expressed in two ways:

- i. as the proportion of paired females carrying foetuses (live or dead) at the time of dissection, and
- ii. as the proportion of paired females, which had vaginal plugs, carrying foetuses (live or dead) at the time of dissection.

It has been reported that many females in high-density mouse populations become pregnant, only to lose their litters early in the pregnancy (Retzlaff, 1938; Christian and Lemunyan, 1958; Lloyd and Christian, 1969). Other experiments have shown that moderate crowding (groups of 4 and 8 mice) increases fertility

(Southwick and Bland, 1959). It has also been observed that increasing population density causes a rising plug:pregnancy ratio in mice (Crew and Mirskaia, 1931).

In the present study, there were no significant differences in fertility rates between the groups.

As it has been shown that implantation may fail when a mated female mouse is exposed to a new male (Bruce, 1959, 1960, 1961), this might well explain the reduction of fertility with increasing population density reported in the literature for breeding groups containing more than 1 male, but not seen in the present study.

Indeed, it has been reported that the same numbers of litters per female are born in permanently mated pairs, trios (2 females and 1 male) and quartets (3 females and 1 male) (Bruce, 1954), and even postulated that the presence of other females may help towards stabilizing pregnancy in mice (Bruce, 1960).

4.1.4 Bodyweight of 14-days-pregnant mice (Fig. 3)

The effect of group size and thus of population density upon weight gain has been reported more often for male mice than for females. While some authors have shown that the growth rate of males kept in isolation is better than for grouped males (Christian, 1955a, 1955b; de Feudis, 1974), others have failed to demonstrate this difference (Anton, 1969; Gärtner and Bonath, 1971) or have reached the opposite conclusion (Vetulani, 1931). It has been suggested that the environmental temperature may play a role in deciding whether isolation or grouping leads to the better growth rates (Retzlaff, 1939; Rabasa, 1952).

As far as female mice are concerned, Weltman et al (1968) found better growth rates for mice kept in groups of 2 than for mice kept in isolation, while Anton (1969) found no difference between female mice kept in isolation or in groups of 7 or 28, this larger group size representing an extreme population density of

16 cm² per mouse.

In the present study, no significant differences in bodyweight between the groups were detected.

4.1.5 Uterus weight (Fig. 3)

There was no evidence that uterus weight at 14 days of pregnancy is influenced by cage size, group size or population density. As uterus weight is essentially a combination of the two parameters number of fetuses and average fetus weight, it will not be discussed in detail here.

The uterus weight expressed as a % of the bodyweight was remarkably constant in all groups.

4.1.6 Weight of ovaries (Fig. 4)

While it has been reported that increasing population density leads to a reduction in the weight of the testes of male mice, both in populations of fixed size (Christian, 1955a, 1955b) and in freely-growing populations (Christian, 1956), other experiments have failed to demonstrate this effect (Southwick and Bland, 1959).

As far as ovary weight is concerned, Brain and Nowell (1970a) found the ovaries of isolated female mice to be heavier than those of grouped animals. Brain (1977) reached the same conclusion after examining lactating female mice which had been isolated or grouped prior to the birth of their litters.

In the present study, there were no significant differences in ovary weight between the groups.

4.1.7 Weight of left adrenal gland (Fig. 4)

Selye (1950) demonstrated that animals responded in a stereotypical manner to a variety of agents including infections, intoxications, trauma, nervous strain, extremes of temperature,

fatigue and irradiation. Although these agents acted in different ways, they possessed a common feature in that they placed the body in a state of general stress. It was proposed that superimposed upon all the specific effects of these injurious agents were the manifestations of the nonspecific stress itself. Selye called this generalized reaction the general adaptation syndrome (GAS), and characterized it as being produced by agents which have an effect on a large portion of the body and as being a state of defence against an injurious agent. Selye described the GAS as having three stages: 1. The alarm reaction in which a generalized initiation of the body's defence mechanism is brought about by a release of adrenocorticotrophic hormone (ACTH) and resultant discharge of steroids. 2. The stage of resistance in which the animal continues to combat the injurious agent while the adrenals accumulate a reserve of steroid. 3. The stage of exhaustion in which the symptoms are similar to the alarm reaction. Manifestations of the GAS include adrenocortical enlargement and hyperactivity, and degeneration of the gonads.

Since then, increased adrenal gland weight and/or increased plasma corticosteroid concentrations have frequently been used to characterize a state of stress in male mice caused by crowding and resulting in reduced testicular weight and reduced fertility. The reader is referred to Christian (1960a, 1963) for comprehensive reviews of this work. Very much less attention has been paid, however, to the effect of population density on adrenal gland weight and function in female mice.

Christian (1956) compared the adrenal gland weights of females from freely-growing populations with those of monogamously paired females. He found that adrenal gland weight increased with population density in females of more than 16 g bodyweight, but not in lighter females.

Anton et al (1968), Bronson and Chapman (1968) and Anton (1969) all reported heavier adrenal glands in isolated than in grouped female mice.

Christian (1960b) and Mody and Christian (1962) found a significant increase in the size of the zona fasciculata of the adrenal gland of female mice with increasing population density. This increase was much less than was observed in males under similar circumstances, and was offset in terms of weight by an involution of the X-zone.

Brain and Nowell (1970a) found that isolated female mice had larger adrenal glands than grouped females, but that this greater weight was not reflected in any increase in corticosterone output in response to ether stress.

An increase in adrenal gland weight is not always accompanied by an increase in corticosterone production (Louch and Higginbotham, 1967).

Increased adrenal gland weight may be due to:

1. Hypertrophy of the active steroid-producing tissue in response to stress,
2. Increase in the amount of inert lipid stored in the gland,
3. Vasodilation of the gland,
4. Increase in the size or content of the medulla,
5. Hypertrophy or vacuolation of non-active cortical tissue,
6. Increased size of the connective tissue capsule.

In such a case, organ weight can only serve as a rough indication of function (Brain, 1970).

Furthermore, it has recently been shown that the adrenal gland of the female mouse reduces in size during pregnancy, while at the same time increasing its output of corticosterone (Brain and Nowell, 1970b; Brain, 1977), and that the increase in weight of the adrenal gland usually seen when female mice are isolated

(Brain and Nowell, 1970a) is abolished during pregnancy (Brain, 1977). This phenomenon appears to be due to the disappearance during pregnancy of the X-zone of the adrenal gland (Chester-Jones, 1952), a region of vacuolated, oestrogen-dependent cells on the border of the medulla and cortex, which increases in weight when non-pregnant mice are isolated, possibly due to increased cell vacuolation (Brain, 1970).

Other methods are available for the estimation of stress states in mice, but they lay outside the scope of this study. They include:

- a) Catecholamine depletion. Welch and Welch (1965) have shown that grouped mice have significantly lower levels of brain norepinephrine than isolated mice, but Anton et al (1968) and Anton (1969) were unable to confirm these findings.

Welch and Welch (1971) have also demonstrated that isolation can lead to a decline of catecholamines stored in the adrenal medulla in comparison with grouped mice.

- b) Leucopenia and eosinopenia. Weltman et al (1968) reported that female mice isolated for 16 weeks showed significant decreases in total leucocytes accompanied by marked eosinopenia, and attributed this to heightened metabolic rates and hyperadrenocortical activity.
- c) Weight of gonads. As only pregnant females were dissected in the present study, uterus or ovary weight could not be used as an indication of stress.
- d) Plasma corticosterone level. The circulating titres of glucocorticoids can be assessed, both 'basal' (unstressed) and/or 'stressed' (following superimposition of standard stresses, such as ether or defeat) (Brain, 1975).
- e) The adaptation times of further physiological parameters after changes in environmental conditions are reviewed by Gärtner and Stoll (1972).

It was decided that adrenal gland weight should be measured in the present study as relatively high population densities were being used, at which the adrenal gland weight might prove useful as an indicator of stress.

No significant differences in the adrenal gland weights between the ten groups were found, neither absolutely nor when expressed as a $^{\circ}/_{ooo}$ of bodyweight.

4.1.8 Number of corpora lutea (Fig. 5)

The number of corpora lutea found in the ovaries on the 14th day of pregnancy was assumed to be identical to the number of eggs ovulated during the oestrous cycle in which the female was mated. Corpora lutea counts were made within 30 minutes of the dissection under a dissection microscope at a magnification of 12x. Only bright pink corpora lutea protruding from the surface of the ovary were counted as corpora lutea of pregnancy (MacDowell and Lord, 1925), to distinguish them from non-functioning, cyclic corpora lutea.

The counts were always performed 'blind' - that is, without checking how many foetuses had been found at the dissection -, and always by the same person (the author).

In 16 of 1030 ovaries thus examined, less corpora lutea were found than implantations in the same horn. While it is known that transuterine migration of fertilized mouse eggs can occur (McLaren and Michie, 1954), it was often the case in the present study that the second horn already had as many foetuses as corpora lutea, so it is assumed that counting errors were responsible for at least some of these discrepancies. This was probably due to 2 corpora lutea occasionally being so close together that they were counted as one (Bradford, 1969).

In the present study, the average number of corpora lutea found in the ovaries of the female mice of all groups was remarkably constant. No evidence was found to suggest that the group size,

cage size or population density influence the ovulation rate of grouped female mice.

The females which mated on Days 1-4 certainly ovulated while they were paired monogamously with the males, so the group sizes and population densities in the group cages could only have exerted an indirect effect at the time of ovulation.

The situation for the female which mated on Day 0 is different. Pairings were established at between 07.30 and 08.00 h, and the vaginal plug examination carried out between 15.30 and 16.15 h. Matings could only have taken place between 07.30 and 16.15 h.

Rugh (1968) has stated that, although oestrus usually begins around midnight with mating most common at about 02.00 h, short exposures of female to male may lead to mating either in the early morning or late evening. Evening matings resulting in fertilization probably depend on the survival of spermatozoa in the female genital tract until the subsequent ovulation which usually occurs between 02.00 and 04.00 h, while morning matings resulting in fertilization depend upon the presence in the ampulla of the oviduct of ova from the previous night's ovulation.

Bronson et al (1966) wrote that such out-of-phase mating is probably restricted to animals that would normally have mated during the previous night, and that it is eggs from the previous night that are fertilized.

It is not possible to state for certain which was the case in the present study. It is therefore not certain whether ovulation occurred while the females were still in their group cages, or not until they had been paired for about 20 hours.

4.1.9 Number of living fetuses, number of dead implants and number of corpora lutea minus total number of fetuses (alive and dead) (Fig. 5)

Several authors have reported that the mean litter size per female mouse at birth reduces with increasing population density (Crew and Mirskaia, 1931; Christian, 1956; Christian and Lemunyan, 1958; Lloyd and Christian, 1969), and that the death rate of fetuses in utero increases as the population density rises (Christian, 1956; Christian and Lemunyan, 1958; Lloyd and Christian, 1969). Retzlaff (1938), on the other hand, repeated Crew and Mirskaia's experiment and concluded that overcrowding does not effect litter size.

Bruce (1947) demonstrated that litter size at birth is the same for monogamously paired females as for females from quartets (1 male and 3 females), while Porter and Festing (1970) found higher mean litter sizes at birth for pairs than for trios (1 male and 2 females), although varying the size of the cage between 407 and 1344 cm² had no effect on litter size. Benhar (1969) found that monogamous pairs had slightly larger litters in cages of 508 cm² than in cages of 406 cm².

For the present study, it was decided not to examine the litter at birth, but to dissect the female during the pregnancy. A true estimation of litter size after birth is not possible, as there is uncertainty as to whether newborn mice have been eaten by the mother or by other animals in the cage. Reliable values for the weights of the newborn mice would also have been lost, because the litters cannot always be weighed immediately after birth as the moment of birth is not always observed. Furthermore, other valuable data such as the weights of the placentae would have been lost.

There was also another reason. It is clear from the literature that the post-natal survival rates of litters of mice decrease

with increasing population density, and that this decrease is due, at least in part, to the presence of other females.

Brown (1953) found that the most important factor directly relating to post-natal litter survival both in mouse populations of fixed size and in growing populations was the condition of the nest at and shortly after parturition. When more than 1 female was present in a cage, he found that other females would often assist the parturient female in the construction of its nest, but that this assistance frequently lead to nest walls being removed or destroyed. He found that at least 1 young mouse was weaned from 82 % of properly constructed nests, while no young were weaned from trampled nests.

Southwick (1955) also reported that nest destruction and abnormal communal nesting both increase with increasing population density, and that both apparently have a detrimental effect on litter survival.

Bruce (1954) bred mice in pairs, in trios (1 male and 2 females) and in quartets (1 male and 3 females). She found death rates from birth to weaning of 25 % for litters born to the pairs, and 34 % for litters born to the trios and quartets.

Porter and Festing (1970) reported pre-weaning mortality rates of 2.9 % for breeding pairs of mice and 12.8 % for breeding trios.

Brain (1977) placed heavily pregnant mice either in groups of 8, in pairs or in isolation. 17 days later, he counted the pups remaining in each litter. There were significantly less survivors per litter in the groups of 8 than in either the groups of 2 or the isolated females.

In view of these findings, it was decided that the present study should examine the effects of group size and population density only upon pre-natal development. The 14th day of pregnancy was chosen as the stage at which the females should be dissected,

this being the earliest stage of pregnancy at which, in a breeding population, females might be identified as pregnant and removed from their groups to bear and raise their litters in isolation.

The results of the present study are shown in Fig. 5. No significant differences in the number of foetuses surviving per group on the 14th day of pregnancy nor in the number of corpora lutea not represented by foetuses could be detected.

4.1.10 Average weight of living foetuses, average placenta weight, sum of weight of living foetuses (=litter weight) (Fig. 6)

Christian and Lemunyan (1958) stated that crowding apparently has no effect on the growth of young mice in utero, although they did find higher pre-natal death rates and slower pre-weaning growth rates in crowded mice compared with isolated mice.

In the present study, no significant differences between groups were found for the average foetus weight, average placenta weight or average sum of weights of living foetuses (= 'litter weight').

The present study failed to reveal a suppression of reproduction due to group size or population density in all-female groups similar to that seen for males in all-male groups, or for both sexes in mixed populations.

Were the group sizes and population densities too low to demonstrate such an effect? This question is best answered by considering the group sizes and population densities in experiments where reproductive suppression has been demonstrated. As far as all-male populations are concerned, Christian (1955a) found depressed reproductive function in groups of 4 (220 cm^2 per mouse) compared with isolated controls.

In breeding populations of fixed size, Crew and Mirskaia (1931) and Retzlaff (1938) reported reduced rates of reproduction in groups of 2 males and 2 females (313 cm^2 per mouse) compared with monogamous pairs. Christian and Lemunyan (1958) found very much higher levels of pre-natal mortality in populations of 10 males and 10 females (73 cm^2 per mouse) than in monogamously paired controls.

In freely-growing populations, Christian (1956) found a steep decline in birth rate when more than 10 adult mice were present (1341 cm^2 per mouse). Maximum population levels and, therefore, depressed birth rates have been observed at 80 weaned mice (122 cm^2 per mouse) (Lloyd and Christian, 1967), at 20 - 60 weaned mice ($145 - 435 \text{ cm}^2$ per mouse) (Lloyd and Christian, 1969) and at less than 60 weaned mice (145 cm^2 per mouse) (Lloyd, 1975). In the present study, group sizes of up to 16 mice and population densities as high as 45 cm^2 per mouse were used, so an effect comparable with that seen in all-male groups or in mixed populations would certainly have been detected.

It is important to differentiate between those parameters which were directly under the influence of the group size and population density, and those which were only indirectly affected. The fertility rates reported in this study were fertility rates for monogamously paired females. It is only possible to say that the prior grouping conditions had no significant effect on fertility. As already discussed, the situation is unclear as far as ovulation, and therefore the corpora lutea count, is concerned.

With the exception of the corpora lutea count, all other parameters assessed in Figs. 3-6 were under the direct influence of the group size and population density.

It can therefore be stated that group sizes and population densities high enough to cause suppression of reproduction in

all-male and mixed groups of mice had no deleterious effects on reproductive parameters during the first 2 weeks of pregnancy in females in all-female groups.

It may be the case that interactions between male mice are primarily responsible for the social stress which leads to suppression of reproduction in mixed populations of mice. As only 1 female per group was mated in the present study, the possibility that interactions between pregnant females generate social stress cannot be ruled out.

What are the practical implications of the findings of this study?

As already discussed in Section 4.1.9, there is no doubt that post-natal litter survival rates decrease with increasing size of the breeding groups, and that this decrease is due in part to the presence of other females. Scientific establishments and commercial breeders often breed using trios (1 male and 2 females), quartets (1 male and 3 females) or even harems, however, as the production per breeding group - but not per breeding female - can in this way be increased. In a breeding period of 16 weeks, Bruce (1954) weaned an average of 33 mice from breeding pairs, 54 from breeding trios (= 27 per female) and 69 from breeding quartets (= 23 per female) housed in cages of identical size. Thus, more litters can be produced per cage, but less mice are weaned per litter. Another method used in practice is to breed in trios, quartets or harems, but to remove heavily pregnant females from their groups and allow them to bear and wean their young in isolation. This method has the disadvantage that the females cannot be mated at the post-partum oestrus, so that the interval between litters is much longer for each female (Bruce, 1947).

However, the present study suggests that it may be possible to avoid compromises of this sort when pregnant mice are required for experimental purposes, as 14-days-pregnant females from

harems should show no suppression of reproductive function in comparison with pregnant females from breeding pairs. The effects of interactions between pregnant females and the presence of a male might, however, influence the development of pregnancy.

More important, the study provides basic data which can be used to assess whether the group sizes and population densities were excessive, that is whether they exerted a negative effect on certain aspects of female reproductive physiology, and thus provide one objective means of assessing whether current thoughts as to maximum group sizes and population densities for female mice are well-founded.

Recently, reference values have been proposed for the floor area requirements of laboratory animals (GV-SOLAS, 1979). According to these reference values:

- the smallest housing unit for a single mouse should have a floor area of ca. 200 cm^2 . In the present study, Group 1 mice were housed singly in cages with a floor area of 180 cm^2 ;
- adult mice in groups of less than 10 should be provided with a floor area of $2 \text{ cm}^2/\text{g}$ bodyweight. Assuming an average bodyweight of ca. 40 g in the present study (i.e. the average of pregnant and non-pregnant mice in each cage), then the mice in Groups 3 and 6 were provided with ca. $1.1 \text{ cm}^2/\text{g}$;
- adult mice in groups of more than 10 should be provided with a floor area of $1.3 \text{ cm}^2/\text{g}$ bodyweight. Assuming a bodyweight of ca. 40 g in the present study, then the mice in Group 10 were provided with ca. $1.2 \text{ cm}^2/\text{g}$

Thus, no suppression of reproduction was seen in pregnant female mice at the population densities recommended by GV-SOLAS.

4.2 Effects of time of day on various parameters (Figs. 7-12)

It has already been noted that male and non-pregnant female F $\ddot{u}$ -Albino mice are significantly heavier in the morning (08.00) than in the afternoon (14.00), despite the continuous availability of food and water (Ellery and Kinnen, 1977).

In the present study, all the females to be dissected on a particular day were kept in groups of up to 6 from 07.00 h until they were dissected. The order in which they were dissected was determined using randomization tables. The majority were dissected during the morning (i.e. up until ca. 11.30 h). Food and water were continuously available up until the time of dissection.

The relation between the time of day and the average bodyweight, uterus weight, ovary weight, adrenal gland weight, foetus weight, and placenta weight are shown in Figs. 7-12 for all 14-days-pregnant females dissected between 07.30 and 11.30 h.

A steady and highly significant decrease was seen in the average bodyweight from 07.30 to 11.30 h, reflecting the results obtained for male and non-pregnant females of the same stock. Despite this, the average foetus weight and average placenta weight showed a significant tendency to rise during the same period, and the ovary weight remained fairly constant. The rises in average foetus weight and average placenta weight were reflected in a significant rise in average uterus weight.

The average adrenal gland weight increased irregularly but significantly with the time.

4.3 Effects of day of pairing on various parameters (Figs. 13-16)

The day after pairing upon which female mice mated had a significant effect on several parameters.

The variation in uterus weight and in the sum of the weights of living foetuses was highly significant, whereby females mated on

the same day as they were paired (Day 0) had significantly lighter uteri and litters than females mated on one of the subsequent four days. This was due particularly to the fact that the average weight of each foetus was lower, and less to the fact that less foetuses were present per litter.

The reason for this finding is probably as follows. As already discussed in Section 4.1.8, Bronson et al (1968) and Rugh (1968) proposed that female mice usually ovulate and mate in the early hours of the morning. In the present study, this could apply to the females mating on Days 1-4 of the pairing period, but not to those mating on Day 0 because they were not paired until 07.30 h in the morning. In other words, the foetuses of females mated on Day 0 were probably some hours younger than those of females mated on Days 1-4, and for this reason weighed less. This aspect should be borne in mind when pairings are made with a view to supplying embryos of a known age for experimental purposes.

Several other significant findings were made. The females mated on Day 4 of the pairing period produced significantly less corpora lutea, living foetuses and total implants than the females mated on Days 1-3. However, as these foetuses were relatively heavy, this was not reflected in significant differences between total litter weights (i.e. the sums of weights of living foetuses). It thus appears that the ovulation rate of females mated on the fourth day after pairing is lower than that of females mated on the previous three days.

The ovary weights and adrenal gland weights of females mated on Day 3 were significantly higher than those of females mated on other days. The reason for these findings is unknown.

5. SUMMARY

3060 28-day-old female F₁-Albino mice were kept in groups of 1, 2 and 4 in Makrolon Type I cages, groups of 2, 4 and 8 in Makrolon Type II cages and groups of 2, 4, 8 and 16 in Makrolon Type III cages (representing population densities of between 45 and 385 cm² per mouse) for a period of 21 days. At the end of this time 1 female per cage (total 600 mice) was removed from its group and paired monogamously with a male mouse of the same stock in a Makrolon Type I cage. All paired females were checked for the presence of vaginal plugs on the afternoon of pairing, and mated females were immediately returned to their group cages. On each of the following 4 mornings, the females which were still paired were re-examined, and those with vaginal plugs were immediately returned to their group cages. Females which had shown no plug up until the 4th day after pairing were also returned to their groups.

14 days after its plug had been found, each of the previously paired females was killed and dissected, the following parameters being recorded: bodyweight, uterus weight, ovary weight, left adrenal gland weight, foetus weights and number of foetuses (live and dead), placenta weights and number of corpora lutea. Paired females in which no plugs had been found were also dissected, but bodyweights, organ weights and foetus and placenta weights were not recorded (unknown duration of pregnancy).

The study detected no effects, detrimental or beneficial, exerted by cage type, group size or population density on the reproductive performance of female mice kept in all-female groups during the first 14 days of pregnancy. It is suggested that the suppression of reproduction due to group size and population density reported in the literature for mixed populations and for all-male groups may be due to interactions between male mice. The results of the study were discussed in connection with the stocking rates recommended for standard cages.

The following two findings were incidental to the theme of the study:

- i) While the bodyweight of pregnant female mice fell significantly between 07.30 and 11.30 h on the 14th day of pregnancy, both the foetus weight and the placenta weight rose significantly during the same period.
- ii) The foetuses of female mice mated on the day of pairing were significantly lighter than those of females mating subsequently during the pairing period, probably due to their being younger at the time of section.

Zusammenfassung

3060 weibliche Fü-Albino Mäuse im Alter von 28 Tagen wurden in Gruppen von 1, 2 und 4 Tieren in Makrolonkäfigen Typ I, von 2, 4 und 8 in Makrolonkäfigen Typ II und von 2, 4, 8 und 16 in Makrolonkäfigen Typ III gehalten. Dies entspricht Populationsdichten von 45 bis 385 cm² pro Maus. Nach 21 Tagen wurde je 1 Weibchen (total 600 Mäuse) aus ihrer Gruppe entfernt und monogam mit einem Männchen desselben Mausstammes in einem Makrolonkäfig Typ I verpaart. Alle verpaarten Weibchen wurden am Nachmittag des Verpaarungstages auf Vaginalpfröpfe untersucht und die gedeckten Tiere sofort zu den Gruppen, aus denen sie stammten, zurückgesetzt. Jeweils an den vier folgenden Tagen wurden vormittags die noch verpaarten Weibchen wiederum auf Vaginalpfröpfe untersucht und die Tiere mit positivem Befund zu ihren Gruppen zurückgesetzt. Auch Weibchen, welche bis zum vierten Tag nach der Verpaarung keine Vaginalpfröpfe aufwiesen, wurden ihren Gruppen wieder zugewiesen.

14 Tage nach dem Feststellen des Pfröpfens wurde jedes Weibchen getötet und sezirt, wobei folgende Parameter bestimmt wurden: Körpergewicht, Uterusgewicht, Ovariengewichte, linkes Nebennierengewicht, Fötusgewichte sowie Anzahl an Föten (lebend oder tot), Plazentagewichte und Anzahl an Gelbkörpern.

Verpaarte Weibchen, bei welchen keine Pfröpfe festgestellt worden waren, wurden ebenfalls sezirt, jedoch wurden Körper-, Organ-, Fötus- und Plazentagewichte wegen der unbekannten Trächtigkeitsdauer nicht festgehalten.

Bei Mäusen, die in nur Weibchen-Gruppen gehalten wurden, konnten durch diese Untersuchungen weder positive noch negative Auswirkungen auf verschiedene reproduktions-physiologische Parameter festgestellt werden, welche durch Käfigtyp, Gruppengrösse oder Populationsdichte hervorgerufen wurden während der ersten 14 Trächtigkeitstage. Möglicherweise sind Wechselwirkungen zwischen männlichen Mäusen für die in der Literatur beschriebene Unter-

drückung von Reproduktionsfunktionen in gemischten sowie in nur Männchen-Gruppen verantwortlich. Die Resultate vorliegender Untersuchungen wurden im Zusammenhang mit den empfohlenen Besatzen für Standardkäfige besprochen.

Zusätzlich wurden folgende Nebebefunde erhoben:

- i Während das Körpergewicht der 14 Tage trächtigen Weibchen zwischen 07.30 und 11.30 h signifikant abnahm, nahmen die Gewichte der Föten und Plazenten während derselben Zeitperiode signifikant zu.
- ii Die Fötusgewichte der am Tag der Verpaarung gedeckten Weibchen waren signifikant niedriger als bei Weibchen, welche an den darauf folgenden Tagen gedeckt wurden. Wahrscheinlich sind die niedrigeren Fötusgewichte durch deren geringeres Alter am Sektionstag zu erklären.

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